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FACTORS GOVERNING THE INITIATION OF SPROUT GROWTH IN CITRUS SHOOTS*

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I. INTRODUCTION

It is a well known fact that in many plants the removal of a portion of a vertical shoot results in the outgrowth of buds which otherwise would have remained dormant. This outgrowth also occurs when a vertical stem is changed to a horizontal position. This phenomenon is generally termed regeneration or reconstitution. The term regeneration is applied in this paper to the outgrowth of buds when this results either from the removal of a part or from the change of position of a stem.

In most cases this outgrowth on vertical shoots is confined to the buds in the uppermost region, the length of the sprouts declining steadily as the distance from the apex increases. In horizontal shoots the outgrowth is confined to the dorsal side, the buds on the ventral side remaining dormant. This dominance or subordination is commonly referred to as physiological correlation or simply correlation.

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With many plants, when an isolated piece of stem is placed under favorable conditions, sprouts will grow from the apical and roots from the basal end. This type of response is supposed to be due to polarity of the stem.

The investigations here reported deal with regeneration of vertical and horizontal Eureka lemon (*Citrus Limonia* Osbeck) shoots and Chinese lemon (*Citrus Medica* var.) cuttings. Both species are evergreens and their growth habits are similar. The tendency of young trees, or of old trees which have been pruned severely, is to produce long, succulent, vertical shoots. So long as the tip grows vigorously these shoots remain unbranched but when, toward the end of the growing season, the rate of growth decreases, laterals appear near the apex. If a shoot is bent permanently into a horizontal position, laterals are produced along the dorsal side. The shoots of both species exhibit very strikingly the phenomenon of polarity in regeneration, hence this material was used for the purpose of studying the factors involved.

II. THEORIES OF REGENERATION

Before proceeding with an account of these investigations it may be profitable to outline the outstanding hypotheses which have been advanced to explain the phenomena mentioned above. Vöchting[23] concluded from his extensive studies that polarity in vegetative shoots is primarily due to unknown internal influences. External factors like gravity, light and moisture may modify polarity to a limited extent but the internal influences persist from generation to generation.

Sachs[22] explained polarity by postulating the existence in stems of shoot-forming substances that migrate upwards and root-forming substances that migrate downwards.

Loeb[13] explained the results of his earlier experiments with *Bryophyllum calycinum* in the sense of Sachs' theory, namely that the "flow of (specific?) substances in the plant determines when and where dormant buds or anlagen shall begin to grow."

From later studies,[14] however, Loeb concluded that there is an inhibitory substance produced at the stem apex which flows in the direction of the basal buds, and he believed that the reason the uppermost bud grows out first when the stem is cut from the mother plant is that this bud is first freed from inhibitory substances.

Subsequently Loeb practically renounced the hypothesis of an inhibitory substance and concluded[16] on the basis of other experiments with Bryophyllum that "a simple mass relation can be used as a guide through the bewildering maze of the phenomenon of regeneration." This mass relation is that equal masses of isolated tissue of the same type exposed to the same external factors produce about equal masses of regenerated roots and shoots in equal length of time. The inhibiting action of one part of a tissue upon another he explained by stating that the sap starts to flow to the group of bud anlagen which begin to grow first and that the other part of the tissue remains dormant because of the continuous flow of sap to the growing part. Loeb mentioned two possible explanations of polarity, first, that there might be a chemical difference between ascending and descending sap which determines the nature of growth and, second, that the anlagen reached by the ascending and descending sap are primarily different. He considers the second alternative the most plausible since he was able to get regeneration of roots with both ascending and descending saps.

Goebel[7] explained that the reason why regeneration takes place when a stem is cut is that the nutritive materials accumulate below the cut.

Curtis[5] suggested that "inhibition of shoot growth at nodes below the terminal one may be due to a lack of sufficient food and to inability to compete successfully for water rather than to a backward flow of some inhibitor." In a previous paper[4] he reported results with sucrose solutions which he cites as an example of reversal of polarity. On the basis of unpublished results he claims to have found other substances which are even more efficient than sucrose in the reversal of polarity.

Robertson[21] believes that the growth-influencing substance concerned in regeneration is the nuclear autocatalyst, which is formed in the cell nucleus and escapes into the pericellular fluid during mitosis. In any given cell, division is regulated by the relative volumes of nucleus and cytoplasm or by what he terms the nuclear-cytoplasmic ratio. Every cell then contains endogenous autocatalyst which is retained within the nucleus and exogenous autocatalyst which has escaped into the pericellular fluid. He shows mathematically that if the exogenous supply is large, then the endogenous supply is small and vice versa. Hence if the supply of exogenous autocatalyst is large, only a small supply of endogenous autocatalyst can be produced before equilibrium is imposed upon the reaction of

nuclear synthesis, and if this does not lead to cell division then no further multiplication of this type of tissue is possible. But if the supply of exogenous autocatalyst is small, then the endogenous autocatalyst can escape into the pericellular fluid and renewed synthesis of nuclear material becomes possible.

It will be seen that Robertson supports the general theory held by Sachs, Goebel, Loeb, etc., that polarity is brought about by the transportation of actual substances. His own theory, however, is more specific than those advanced by others like Sachs and Loeb, for he actually ascribes the rôle of inhibitor and accelerator to one and the same substance, the nuclear autocatalyst.

Child[3] believes that the theory of formative stuffs or transportative correlation has little real explanatory value. He does not deny the existence of transportative correlation but he maintains that it cannot exist until the different systems are present; for example, the flow in opposite direction of shoot and root-forming substances as postulated by Sachs cannot occur autonomously but becomes possible only when regional differences of some sort are present which determine the flow. Child assumes that dominance is effected by the transmission of energy-changes or excitation, rather than by the transportation of chemical substances. This assumption is based on his theory which will now be considered.

In all organisms there are gradations in the intensity of metabolic processes which determine the fundamental outlines of axial symmetry and structural differentiation. Centers of high metabolism like the head of a planarian worm or the apex of a stem tend to dominate or control centers of low metabolism. Dominance then depends primarily upon the rate of metabolism and seems to operate by impulses, excitations, or changes transmitted in various ways from the dominant region to other parts. Although the primary difference between dominant and other levels of the gradient is purely quantitative, yet quantitative changes may, sooner or later, bring about differences in constitution and character of the protoplasmic substratum. Each level of the gradient develops a characteristic protoplasm and the character of the protoplasm in turn alters or modifies the characters of the reaction. In this way, different specific substances may be produced at different levels of the gradient and chemical transportative correlation then becomes possible. Gradients may be reversed or obliterated or new gradients established by environmental agencies which change the metabolic rate in different parts of the organism, but the gradient once established, persists through asexual and perhaps also through sexual reproduction.

In an earlier publication[2] Child discussed the relation of dominance and subordination between different parts of the root system. The results of certain experiments with roots obtained by Goebel and McCallum indicate to him that "not only does a relation of dominance and subordination exist between the different parts of a root system, but that the root system as a whole dominates the stem to a certain extent, so far as the production of roots is concerned. If this dominance and the dominance of the stem-tip both result from metabolic gradients, then there must be in plants possessing roots two metabolic gradients in opposite directions, the apical region of one being in the stem-tip or tips, that of the other in the root-tip or tips." This is impossible unless the two gradients have different paths of transmission or are of different metabolic character. Child therefore conceived the possibility that the inhibiting influence of the roots upon the stem may be a transportative rather than a transmissive correlation and that it becomes ineffective when this transportation decreases to a certain minimum or when the two parts are separated. But the apparent dominance of the root system over the aerial part of the plant with respect to root formation is a secondary relation and hence, according to Child, is dependent upon the primary relation which is transmissive in character.

III. APICAL DOMINANCE IN VERTICAL SHOOTS AND CUTTINGS

A vigorous vertical lemon shoot generally remains unbranched during the entire growing season. If laterals are produced they are mostly confined to the apical region. When such a shoot is cut back to the mature wood, laterals will appear only from buds close to or immediately below the apex of the remaining portion. The number of sprouts produced depends upon the vigor of the mother shoot but ordinarily not more than seven are formed. At first these sprouts grow at about the same rate, but within a few weeks the growth rate of those nearest to the apex is accelerated while that of the subapical sprouts slows down; generally growth ceases after the end of the first cycle. Every healthy bud below this active region can be forced to produce a vegetative sprout by notching or girdling above the bud or by mechanically preventing the apical portion of the shoot from producing sprouts.

The investigations here reported were undertaken for the purpose of studying apical dominance in the Citrus stem. For the work with cuttings the Chinese lemon was chosen because it can be grown more successfully by this method than other species of Citrus. The investigations in the orchard were carried out with shoots of the Eureka lemon. All material was obtained from trees growing on the grounds of the Citrus Experiment Station, Riverside, California.

(*a*) Chinese Lemon Cuttings

In the course of preliminary experiments it was found that if the upper half of a cutting (about 30 cm. in length) was enclosed in a plaster cast and suspended in moist air, sprouts would appear immediately below the cast. When the plaster cast was removed and the cutting again suspended the uppermost buds grew out, while within a few weeks the original sprouts died. The results of this experiment were discussed by Reed and Halma[20] and were considered to support the inhibitor theory advanced by Loeb.[14] The results of the following experiments, however, make it evident that the initiation of sprout growth cannot be explained on the basis of the inhibitor theory.

Chinese lemon cuttings, each possessing ten buds, were divided *into three sets. In one set of 31 cuttings the cut surface and the three* uppermost buds were wrapped tightly with rubber tape (bricklayer's tape); in another set of 31 cuttings the cut surface and the five uppermost buds were wrapped. A set of 15 unwrapped cuttings served as a control. The pressure of the wrapping with tape mechanically prevented development of the buds. The cuttings were then planted vertically to a depth of two to three centimeters in flats containing washed river sand and placed in the greenhouse. In order to increase the humidity of the surrounding air the flats were covered with glass cases.

Measurements of the sprouts produced by each cutting were made every three or four days. The tape was removed when the total length of the sprouts produced by the free portion had reached various lengths. After growth ceased the green weight of sprouts and roots was determined. The data obtained are given in tables 1 and 2 and they are graphically represented in figure 1.

TABLE 1

CHINESE LEMON CUTTINGS; SHOWING THE EFFECT OF TAPING THE THREE UPPERMOST BUDS

Cutting No.	Total length of subapical sprouts		Total length of apical sprouts, cm.
	When apex was freed, cm.	At end of experiment, cm.	
5	0	22	8
24	1	26	7
19	2	22	12
31	3	29	8
15	3	24	12
21	4	21	8
12	4	25	11
25	6	17	22
26	6	17	3
30	7	16	12
16	7	20	14
6	8	21	13
23	8	19	30
17	9	20	5
11	9	23	21
28	10	16	9
22	10	23	7
20	10	17	10
7	11	20	9
27	11	22	9
13	13	28	7
18	14	22	9
29	14	19	6
8	16	18	5
10	16	18	6
1	16	19	24
14	16	23	11
2	17	20	1
4	17	18	16
9	18	31	8
3	21	21	6

TABLE 2

CHINESE LEMON CUTTINGS; SHOWING THE EFFECT OF TAPING THE FIVE UPPERMOST BUDS

Cutting No.	Total length of subapical sprouts		Total length of apical sprouts, cm.
	When apex was freed, cm.	At end of experiment, cm.	
18	1	19	15
21	1	12	10
22	2	17	10
31	2	19	11
29	2	18	13
27	2	13	7
24	2	18	11
10	2	19	19
16	3	18	12
6	4	21	18
26	4	14	9
23	4	23	20
20	4	27	14
12	5	18	11
15	5	13	8
25	5	16	36
28	5	14	15
30	5	14	29
17	6	19	10
5	6	24	14
4	7	18	7
19	8	20	12
11	10	22	7
8	10	18	12
7	11	27	4
13	11	13	9
14	13	15	10
9	18	23	12
1	19	22	8
2	23	25	24
3	26	34	29

In contrast to the results obtained in the experiment mentioned previously,[20] none of the subapical sprouts died. It is important to mention that the control cuttings began to grow from two to three days earlier than the other two sets.

It should be noted that even where no measurable growth was made by the free portions of the cuttings before the tape was removed the ultimate growth compared favorably with that made by other subapical regions which had a better start.

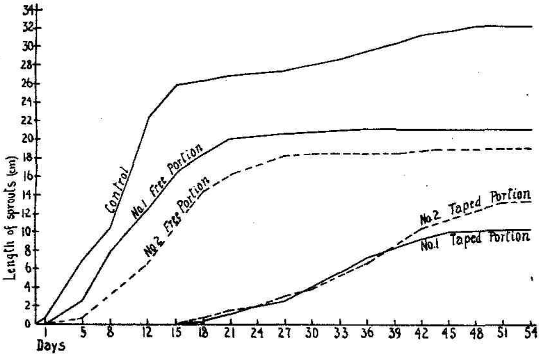

Fig. 1. Chinese lemon cuttings. Average total sprout growth per cutting. No. 1, three uppermost buds taped; No. 2, five uppermost buds taped. Control not taped.

Figure 1 shows that the growth of both free and inhibited portions of the cuttings follows the characteristic S-shaped curve. This suggests the possibility that the two portions, although connected morphologically, behave as two physiological units.

Before examining this possibility it is necessary to discuss the relation between size of cutting and amount of sprouts produced. This is of importance to the interpretation of the data obtained with taped cuttings. During the winter 58 cuttings of various sizes were made from the mature part of 14 mother shoots of the Chinese lemon. They were grown about the same time and under the same conditions as the cuttings in the experiment described above. For the purpose of finding out whether or not a difference existed between apical and basal portions of the mother shoot, the cuttings from each were kept in the order of their position on the mother shoot.

TABLE 3

CHINESE LEMON CUTTINGS; RELATION BETWEEN SIZE OF CUTTING AND AMOUNT OF SPROUTS PRODUCED FROM INDIVIDUAL CUTTINGS OF FOURTEEN MOTHER SHOOTS

Weight = total green weight in grams of sprouts per 100 gr. of green weight of cutting.

Length = total length in cm. of sprouts per 100 sq. cm. of area of cutting.

In the "Individual" column, the apical cutting is given first.

Weight		Length	
Individual	Average	Individual	Average
20.0		81.2	
30.4		64.7	
31.5		54.2	
37.5	29.9	61.6	65.3
35.2		51.1	
27.6		73.8	
32.6	31.8	60.4	61.7
45.3		71.4	
43.9		90.9	
43.1	43.1	80.0	80.7
35.6		70.0	
35.7		81.1	
47.4	39.9	73.1	74.7
29.4		52.6	
24.5		68.1	
20.7		62.9	
30.6		43.3	
27.1	26.5	38.0	53.0
44.2		71.1	
47.3		69.0	
47.9	46.4	76.0	72.0
38.9		55.1	
26.3		72.7	
22.9		35.1	
36.9	31.2	73.9	59.2
36.1		62.4	
27.8		80.0	
28.4		68.8	
25.0		69.0	
22.5	28.0	62.5	68.5
28.0		70.3	
34.2		57.8	
25.5		68.1	
25.8		68.1	
38.9	30.5	59.5	64.8
36.4		70.3	
33.3		52.6	
31.3		56.8	
33.3		55.1	
35.3	33.9	58.4	58.6
54.1		62.5	
36.7		69.9	
49.3		73.4	
41.1		73.8	
40.1	44.3	61.2	68.2
46.8		74.6	
39.1		73.3	
38.3		71.4	
44.0	42.0	66.8	71.5
43.1		83.3	
48.3		66.6	
47.4		62.0	
46.8		68.5	
38.5	44.8	57.1	67.5
43.1		55.1	
34.5		48.9	
34.7		60.0	
29.4	35.4	61.2	56.3

The green weight and length of the sprouts were determined after six weeks when growth had stopped. In table 3 the total length of sprouts is given on the basis of 100 sq. cm. of stem area of the cuttings on which they grew and the green weight of sprouts on the basis of 100 grams of green weight of stem of the cuttings on which they grew. The average green weight of sprouts produced by each set of cuttings varied from 26.7 to 46.4 grams—a difference of *19.7 grams.* The maximum variation in sprout production among the cuttings from the same mother shoot is *17.5 grams* and in the entire set of 58 cuttings is *34.1 grams.*

The correlation coefficient between green weight of cuttings and green weight of sprouts produced is .806 ± .032 and that between stem area and length of sprouts is .747 ± .040. The inclusion of the roots does not alter these values materially.

With these relations in mind we shall now examine the sprout production by the free and the temporarily inhibited portions of the cuttings. Table 4 gives the average amount of growth produced by the free and the inhibited part of the cutting respectively. It will be seen that in the case of cuttings whose uppermost three buds were taped the free parts produced 25.0 grams of sprouts and the inhibited part 42.7 grams—a difference of *18.7 grams.* It was stated above that the greatest difference between cuttings of the same mother shoot was *17.5 grams* and for the entire lot *34.1 grams* (table 3). In view of the fact that the cuttings wrapped with tape were parts of many mother shoots it is evident that, had the two portions of the cuttings been separated, a similar variation in the production of sprouts would have occurred. It is therefore probable that the difference between free and taped portions was due to variation of the material.

TABLE 4

CHINESE LEMON CUTTINGS; MASS RELATION OF SPROUTS ON TAPED AND FREE PORTIONS AS COMPARED WITH SIMILAR RELATIONS ON CONTROLS

Number of buds temporarily taped	Free portion		Taped portion		Entire cutting	
	Green weight of sprouts (grams) per 100 grams of green weight of stem	Length of sprouts (cm.) per 100 sq. cm. of stem area	Green weight of sprouts (grams) per 100 grams of green weight of stem	Length of sprouts (cm.) per 100 sq. cm. of stem area	Green weight of sprouts (grams) per 100 grams of green weight of stem	Length of sprouts (cm.) per 100 sq. cm. of stem area
3 (31 cuttings)	*25.0±1.21*	65.6±1.85	*42.7±3.39*	63.7±3.89	*28.7±1.23*	65.4±1.56
5 (31 cuttings)	36.3±1.35	62.4±1.75	32.6±2.56	65.1±3.17	33.4±1.51	62.9±1.94
Control (15 cuttings)	....	....	....	....	27.7±1.52	59.1±1.20
Control (58 cuttings)	....	....	....	....	35.8±0.75	65.1±0.95

The cuttings whose five uppermost buds were temporarily inhibited produced 36.3 grams from the free portion and 32.6 grams from the inhibited portion—a difference of only *3.6 grams.* As regards length of sprouts produced the values for both sets of cuttings are so close that no explanation is needed.

The figures in table 4 indicate that temporarily inhibiting a part of the apex resulted in the division of the cutting into two physiological units each of which produced sprouts in proportion to its mass of the cutting. If this is correct then the ratio of sprouts to unit weight or area of stem should be approximately the same for

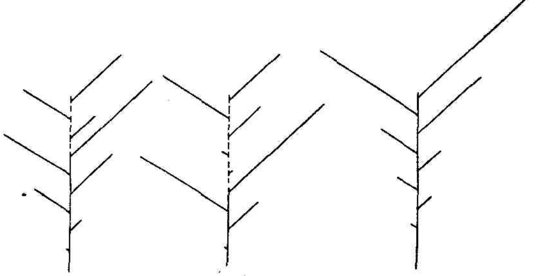

Fig. 2. Chinese lemon cuttings. Comparative length and distribution of sprouts. Dotted line represents the part of the cutting which was taped.

each set regardless of the number or location of the sprouts. This is actually the case. For example, the cuttings whose three uppermost buds were taped produced 28.7 grams of sprouts per 100 grams of cutting, the other set 33.4 grams, and the two sets of controls 27.8 and 35.8 grams respectively (table 4).

Further evidence that we are here dealing with two physiological units resides in the fact that only the uppermost buds of each part produced sprouts. In 30 per cent of the cuttings whose upper three buds were taped the third bud remained dormant when the tape was removed; whereas in the other set only 6 per cent of the cuttings produced sprouts from all five buds and as a rule the fourth and fifth bud remained dormant. This was still more marked in another experiment to be mentioned later. The average distribution and length of sprouts on the average cutting is presented diagrammatically in figure 2.

The question arises as to whether or not these observed facts shed any light upon the nature of the polar character of regeneration in stems. There is no doubt that the growth of the cuttings under the conditions of the experiment depends upon the material stored in the stem, and that this supply becomes exhausted regardless of the number or position of the sprouts produced. According to Loeb[16] the sprout or sprouts which grow out first attract all the available material, hence the other buds remain dormant. The above investigation shows clearly that this is not true for Chinese lemon cuttings, because in many cases the sprouts below the inhibited portion were of considerable length before the tape was removed and yet growth was not prevented in the apical region.

The interpretation of the results of earlier experiments[20] was based on the assumption that an inhibitory substance, produced by the growing apical sprouts, passed toward the basal part of the cutting and thus inhibited the development of sprouts in that region.

From this experiment, it is obvious that that assumption was inadequate, because in this case the apical region of the cuttings had no sprouts to produce an inhibitory substance and, furthermore, when apical sprouts were produced, they were unable to suppress the growth of subapical sprouts.

If we assume that dominance is due to an axial gradient of metabolism declining steadily with the distance from the apex, then a lemon shoot ought to produce a gradation of sprouts from apex to base. This gradation however appears only in a part of the shoot. Furthermore, less growth ought to be produced in the subapical than in the apical region. This was not the case with taped cuttings, where both the upper and lower portions produced growth in proportion to their mass. Table 3 also shows that there is no consistent difference in the weight or length of sprouts produced by the different parts of the mother shoots.

A possible explanation of these experiments is based upon the view held by Curtis[5] that some substance necessary for growth passes upward through the phloem. On this assumption, in the control cuttings the growth promoting substance would move upward until it reaches the uppermost bud or buds which are in condition to make use of it.

But cuttings planted upside down also produce sprouts from the apex, hence we cannot say that this substance can move only upward. We have seen that in the free portion of the taped cuttings sprout growth started later than in the apical portion of the control

cuttings. This time factor is significant. We may assume that the transformation of food reserves into growth-promoting substances is a gradual process which begins at the apex. This view is strengthened by the fact that when only the three uppermost buds were inhibited, the delay in the outgrowth of buds immediately below the tape was not so great as when the five uppermost buds were inhibited.

No definite reason can be given as to why the transformation of food reserves into growth-promoting substances should begin at the apex. Evidently it takes place just as quickly in taped as in untaped cuttings for sprouts will break through weak places in the tape before there is any sign of growth below the tape.

The results obtained warrant the assumption that the earlier release from dormancy of the buds in the apical region is due to the gradual transformation of food reserves into growth-promoting substances from apex to base. The dormancy of subapical buds may be assumed to be due to the ability of the actively growing apical sprouts to draw on the entire supply of growth-promoting substances as fast as they are formed. If the growth of apical sprouts were dependent solely upon the supply of these substances which is present in that region, then buds all along the cutting would have to grow out in order to account for the mass relation obtained.

It is evident from table 4 that the growing sprouts below the taped portion cannot draw on the supply of growth-promoting substances which are stored up in the apical part.

It seems to the writer that the above explanation is more plausible than that based on the downward flow of some inhibitor. A recent investigation by Gardner[6] also indicates that nutritive factors are involved in the initiation of sprout growth.

(*b*) Undetached Eureka Lemon Shoots

Before proceeding with this discussion, it will be profitable to see whether or not the foregoing concept can also be applied to the behavior of attached shoots under orchard conditions. For this purpose the following experiment was made. At the beginning of the growing season, upright, unbranched, one-year-old Eureka lemon shoots were cut back to stubs 50 cm. in length. This left about 24 buds on each shoot. On one set of 28 shoots one-fourth of the uppermost buds were wrapped with rubber tape and on another set of 28 shoots, one-half were so treated. As in the case of the cuttings, sprouts appeared from the free portion. On a certain number of

shoots in each set the tape was removed when at least one of these sprouts reached a length of 5, 15, and 30 to 40 cm., respectively. Twenty untreated shoots served as a control.

Measurements of the length of each sprout produced were made weekly throughout the growing season. A few shoots were taped to the last bud, the intention being to release the buds one by one as sprouts developed.

The results obtained are similar to those obtained with Chinese lemon cuttings. Tables 5 and 6 show that in no case was growth of the free region suppressed after unwrapping the apical portion. Figures 3, 4, 5, and 6 are representative graphs of the growth curves of the free and taped portion. They show with one exception that the growth curves of the two portions run approximately parallel. This exception occurred with shoots whose upper one-half was freed when at least one subapical sprout was 5 cm. in length. Here the growth of the apical portion slightly exceeded that of the subapical one, but not until after the latter had completed its first growth cycle. On the average the control shoots produced sprouts from the uppermost seven buds. The taping of one-fourth of the shoot included from five to seven buds, hence some of the buds below the tape would have produced sprouts without inhibition of the apical portion. But when the taping involved one-half of the shoot, which included from ten to twelve buds, the subapical sprouts were outside of the normally active region.

In discussing the behavior of Chinese lemon cuttings it was emphasized that the difference in the length of time required for apical and subapical buds to produce sprouts may be a factor in regeneration. This time factor was very much more marked in lemon shoots. The subapical sprouts of the shoots whose upper half was taped, appeared one to two weeks later than the sprouts on the control shoots. In the other set a delay of two or three days was noticed. The importance of the position of the bud was still more marked when the taping extended to the last bud. In this case it was at least three weeks before a sprout appeared; besides, these sprouts were weak and yellow. No delay in the appearance of the sprouts was observed on shoots which were cut back to 10, 15 or 25 cm.

TABLE 5

EUREKA LEMON SHOOTS; SHOWING THE EFFECT OF TAPING THE UPPER FOURTH

Shoot No.	Total length of subapical sprouts		Total length of apical sprouts, cm.
	When apex was freed, cm.	Final length, cm.	
21	18	363	401
10	19	132	210
23	20	291	106
7	21	152	561
19	22	223	133
16	22	344	175
4	23	678	208
22	24	392	156
12	25	267	152
28	27	213	75
17	30	104	70
25	35	340	48
3	45	199	116
6	49	412	282
11	53	211	65
15	56	179	141
13	60	70	128
5	66	122	135
26	69	314	269
27	70	289	93
18	121	317	256
8	142	449	237
14	149	268	118
9	157	395	266
1	161	356	274
20	161	281	101
2	200	387	42

TABLE 6

EUREKA LEMON SHOOTS; SHOWING THE EFFECT OF TAPING THE UPPER HALF

Shoot No.	Total length of subapical sprouts		Total length of apical sprouts, cm.
	When apex was freed, cm.	Final length, cm.	
9	7	154	306
15	7	72	274
18	9	135	202
11	16	60	113
3	16	223	276
1	19	260	163
10	19	130	151
20	23	566	306
13	28	205	188
5	33	218	141
23	33	487	186
14	33	242	178
2	34	201	159
27	45	239	247
26	48	96	251
25	49	222	271
19	54	137	264
7	62	281	130
16	70	136	83
17	81	309	207
21	83	88	74
28	98	411	462
24	101	400	145

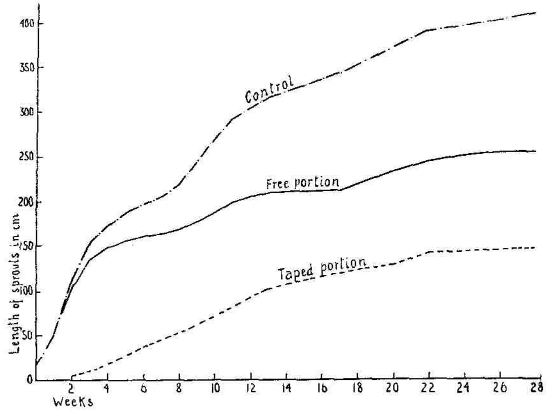

Fig. 3. Vertical Eureka lemon shoots. Average total sprout growth per shoot. Upper fourth taped; freed when at least one subapical sprout was 5 cm. long. Control—not taped.

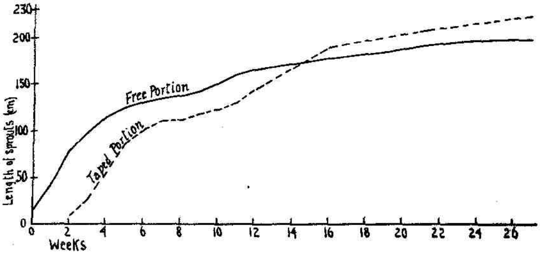

Fig. 5. Vertical Eureka lemon shoots. Average total sprout growth per shoot. Upper half taped; freed when at least one subapical sprout was 5 cm. long.

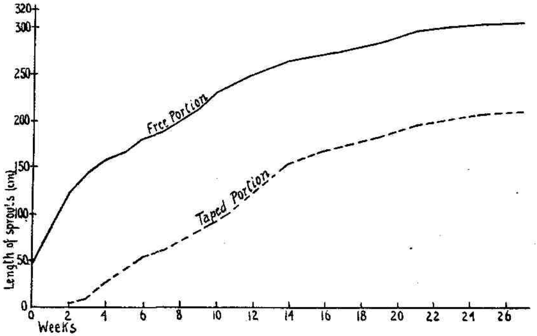

Fig. 4. Vertical Eureka lemon shoots: Average total sprout growth per shoot. Upper fourth taped; freed when at least one subapical sprout was 15 cm. long.

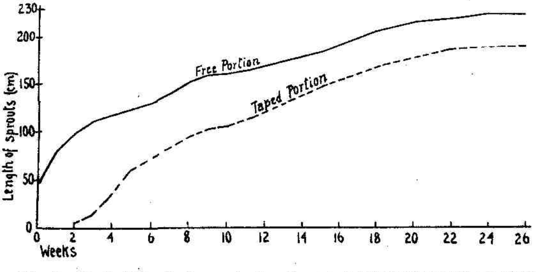

Fig. 6. Vertical Eureka lemon shoots. Average total sprout growth per shoot. Upper half taped; freed when at least one subapical sprout was 15 cm. long.

An important fact, observed also with Chinese lemon cuttings, was the dormancy of certain buds which under normal conditions would have grown out. Figure 7 showes diagrammatically the average length and distribution of the sprouts on the three sets of mother shoots. It is seen that after the freeing of the upper five taped buds, only three grew out, while nine sprouts were produced by the lower untaped portion. In the other set only six out of ten buds grew out in the upper portion while nine sprouts were produced by the lower portion. The average number of sprouts produced was twelve per shoot in the former taped set, fifteen in the latter, and eight in the controls. The difference in the number of sprouts or in their size previous to unwrapping the apex had practically no effect upon the total amount of growth (table 7).

TABLE 7

EUREKA LEMON SHOOTS; SHOWING THE EFFECT OF TAPING THE UPPER FOURTH AND UPPER HALF

Length in cm. per 100 sq. cm. of bark area of shoot.

Upper fourth of mother shoot taped

Average total length of sprouts produced by			
Free portion		Apical portion (final length)	Entire mother shoot
When tape was removed	Final length		
18	186	325	221
29	197	406	249
97	158	371	251
Upper half of mother shoot taped			
15	200	224	213
42	220	187	203
67	255	200	228
	Control		
....	....	....	203

In order to test the reliability of these results the following experiment was made. At the beginning of the growing season 14 pairs of uniform unbranched, one-year-old shoots, situated similarly on the trees, were cut back to the mature wood. On one shoot of each pair, notches were made in the bark just above many of the subapical buds, in order to force out a greater number of sprouts. The shoots thus treated produced about twice as many sprouts as the untreated shoots. At the end of the growing season the sprouts on both sets of shoots were measured and their dry weight determined. The

results are summarized below. The figures are based on 100 sq. cm. of shoot area.

	Total length of sprouts	Dry weight of sprouts
Notched	207 cm.	30 grams
Control	219 cm.	35 grams

The ratio between dry weight of mother shoots and dry weight of sprouts produced was found to be as 1 to 1.8 for each case.

The evidence for vertical lemon shoots is fairly conclusive that the amount of growth produced is approximately proportional to the size of the mother shoot regardless of the number or distribution of the sprouts. It may be mentioned here that a similar relation exists in horizontal shoots.

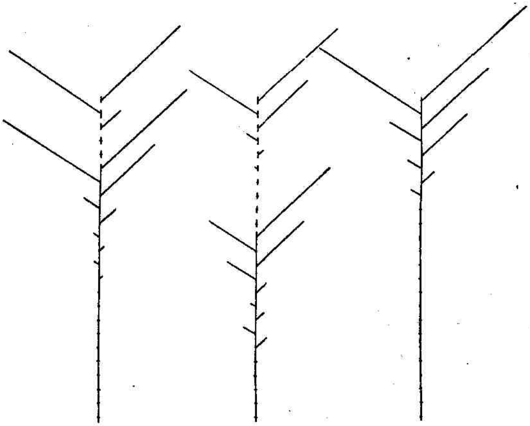

Fig. 7. Vertical Eureka lemon shoots. Comparative length and distribution of sprouts. Dotted line represents the part of the shoot which was taped.

(c) Discussion

The results obtained with lemon shoots corroborate in a general way those obtained with Chinese lemon cuttings. When the outgrowth of buds in the upper one-fourth or one-half is prevented, the parts behave as separate physiological units. A few remarks concerning the cyclic growth of lemon sprouts may be made. Normally

not more than three cycles are made by the sprouts nearest the apex; those further down usually make only one or two cycles. The growth made during the greater part of the first cycle probably depends entirely upon the reserve material stored in the shoot. For the first few weeks all sprouts grow at approximately an equal rate, then the apical ones gain the ascendency. During the second and third cycles elongation is practically confined to a few apical sprouts.

For the purpose of our discussion we may divide the seasonal growth into two periods, one during which growth probably depends on the stored material and the other during which nutrients are supplied. The first period, during which sprout growth is initiated, *is the phase with which we are mainly concerned at present.*

In the shoots the transformation of food reserves into growth-promoting substances is probably a gradual process which begins at the apex. As is the case of cuttings, the growing apical sprouts appropriate the supply of growth-promoting substances as fast as it is formed, hence the buds farther down remain dormant.

The fact that the time required for subapical sprouts to grow out is in proportion to the length of the portion which is taped, suggests the possibility that the formation of growth-promoting substances begins at the apex and in the cuttings this formation takes place whether or not the upper portion is taped. Evidently the growing subapical sprouts can draw on the supply of growth-promoting substances only from below the point of origin of the sprout.

During the second period only a few apical sprouts continue to elongate while the others below make no further growth. The amount of raw materials furnished each shoot depends upon the size of the root system and the number of growing tips. Hence it is conceivable that under favorable conditions each growing tip receives its proportion of this material. But this does not explain why it should only benefit a few of the uppermost sprouts.

A possible explanation is that during the second period the rapidly growing sprouts exert an inhibiting influence upon the growth of the subapical sprouts. This influence, as Loeb's[14] results suggested, may be due to an actual substance (a hormone) which is produced in the growing tip and which, as it travels downward, prevents further elongation of subapical sprouts. Reed[17] found evidence in pruned pear shoots which he considered a confirmation of Loeb's inhibitory hypothesis. In subsequent studies Reed[18, 19] applied this concept to explain the behavior of apricot shoots and of organisms in general.

Barker and Lees,[1] working with pear shoots, came to the conclusion that in addition to the action of an inhibitor other factors such as temperature, bud strength, root action and variety influences determine the growth of buds on the mother shoot.

A general objection to the inhibitor theory is that raised by Harvey.[10] He and others argue that if such a substance is produced in the dominant region, growth should be depressed there also because it would be in greatest concentration there. This, however, would not be true if the inhibiting substance is carried away as fast as it is formed. Since nothing is known about the nature of this substance the objection cannot be met unless we accept Robertson's hypothesis.[21]

It is the writer's view that the apical dominance in attached lemon shoots is governed by two distinct processes. During the initial period the release from dormancy of the buds and sprout growth is governed by growth-promoting substances. Later apical dominance may be ascribed to an inhibiting substance which is produced by the rapidly growing apical sprouts and influence their subapical neighbors. In dealing with cuttings we are concerned only with the release from dormancy.

IV. EFFECT OF INJECTIONS OF VARIOUS CHEMICAL COMPOUNDS ON THE OUTGROWTH OF BUDS

In the preceding part of this paper the view was expressed that the upward movement of growth-promoting substances contained within the cuttings, together with a time factor, are concerned in determining the apical dominance in Chinese lemon cuttings. The next step was to study the effect of substances introduced into the cuttings on the order of bud development and the amount of growth.

Curtis[4] found that treatment with potassium permanganate results in a very marked increase in root growth of various woody cuttings. He also found that immature cuttings produced better root growth when treated with cane sugar. In maturer cuttings this treatment generally resulted in increasing the top growth and in the development of subapical buds which normally remain dormant.

Gardner[6] succeeded in bringing about the outgrowth of basal buds of pear cuttings by introducing a five-tenths per cent solution of sodium nitrate into the basal part of the cutting. Gardner considers it probable that the absorption of sodium nitrate by the basal portion of the cutting lowered the carbohydrate-nitrogen ratio by increasing the nitrogen content.

(*a*) Ligustrum Cuttings

The material was obtained during the winter from a hedge growing in Riverside, California. This plant is an evergreen but goes into dormancy about the same time as the Chinese lemon. Twenty cuttings, each possessing five nodes taken from mature shoots, were used for each treatment, which consisted in the introduction of a solution of a chemical. In ten of them the solution was drawn from base to apex and in the other ten from apex to base. The solution was drawn through the cuttings by means of a filter pump. The cuttings were removed after at least several cubic centimeters of the solution had collected above. They were then thoroughly rinsed with water, and the part which stood in the solution and to which the tubing was attached was cut off. The cuttings were planted vertically in flats containing river sand and kept in a basement room in which a temperature of 25° to 27° C. and a humidity of about 75 per cent were maintained. Light was furnished by a 200-Watt Mazda daylight bulb placed about one meter from the cuttings. Evidently the amount of light was insufficient as none of the cuttings produced roots. Some cuttings treated similarly and exposed to sunlight produced roots but the twig growth was similar to that made in the culture room. Table 8 gives the summary of the results. The manner in which the solution was drawn through the cutting had no effect upon growth, hence no separate data are given.

The important point is that none of the compounds used stimulated greater growth than distilled water. It is remarkable that the acids and bases did not injure the cuttings to a greater extent. It will be noticed that some cuttings died in every lot. Treated with M/5 $Ca(NO_3)_2$ only two cuttings failed to grow while, with the weaker solution sixteen died and the growth was also poor. It is worthy of remark that in most cases more cuttings died with the weak than with the strong solutions. The reason is not evident.

It was observed that in no case was the order of bud development reversed. The greatest deviation from the normal was found in cuttings which received M/5 $Ca(NO_3)_2$ solution. The fact that this compound did not increase growth would indicate that it interfered with the upward movement of growth-promoting substances in the uppermost buds.

Since no roots were produced, Curtis' findings in regard to the root-stimulating effect of potassium permanganate could not be verified. Under the conditions of the experiment both concentrations

gave poor top growth. A few cuttings treated with this compound and placed in sunlight did not give better results than those treated with sugar or distilled water.

TABLE 8

LAGUSTRUM CUTTINGS INJECTED WITH VARIOUS SOLUTIONS, AND GROWN IN SAND

Treatment	Number of cuttings		Total average length of twigs per cutting in cm.	Order of best growth	Average number of nodes which grew
	Dead	Growing			
M/5 HCl	4	16	8.9	7	2.1
M/25 HCl	10	10	7.0	11	1.5
M/5 KOH	8	12	7.3	10	1.6
M/25 KOH	7	14	7.7	9	1.7
M/5 NaOH	8	12	6.3	14	1.3
M/25 NaOH	8	12	5.8	15	1.4
M/1 dextrose	3	17	10.7	3	1.5
M/5 dextrose	10	10	8.8	8	1.5
M/10 $KMnO_4$	7	13	7.3	10	1.9
M/25 $KMnO_4$	5	15	6.4	13	1.5
M/5 $Ca(NO_3)_2$	2	18	10.5	4	2.4
M/25 $Ca(NO_3)_2$	16	4	6.5	12	1.4
M/5 $NaNO_3$	9	11	10.3	5	1.7
M/25 $NaNO_3$	10	10	9.1	6	1.8
Ether water	8	12	11.5	1	2.0
Distilled water	7	14	11.4	2	1.6
Control	9	11	9.1	6	1.4

(*b*) MATURE CHINESE LEMON CUTTINGS

Cuttings of this plant grow well under the conditions described above. In a preliminary experiment injected cuttings of uniform size were suspended in saturated air. For each treatment 14 cuttings were used. The following compounds were used in various concentrations: HCl, KOH, NaOH, dextrose, ether-water and distilled water.

None of these solutions had any influence on the order of bud development. The average total length of sprouts produced per cutting is given below. None of the solutions omitted gave better results.

M/25 HCl	5.7 cm.	Ether water	3.0 cm.
M/25 NaOH	5.3 cm.	Distilled water	9.7 cm.
M/25 KOH	4.4 cm.	Control	5.3 cm.
M/1 dextrose	5.7 cm.		

There is no evidence of any effect of any of the injections, except an increase of growth with distilled water, a decrease with ether-water and a probable slight decrease with KOH. There was no deviation from the normal order of bud development.

In another experiment cuttings possessing 7 to 11 buds were made and well sampled so that each lot contained parts of several trees. For each treatment 15 cuttings were used. The method of injecting the solutions and the condition under which the cuttings were grown were the same as those previously described except that the cuttings were placed in sand. Before planting, the green weight and bark area of each cutting were determined. In all cases twig growth ceased after the third week. After the fourth week the twigs and roots were cut off, weighed and measured.

TABLE 9

CHINESE LEMON CUTTINGS INJECTED WITH VARIOUS SOLUTIONS

Green weight of sprouts and roots in grams per 100 grams of green weight of cuttings.

Number of cuttings dead	Treatment	Weight of sprouts	Order of greatest sprout growth	Weight of roots	Order of greatest root growth
1	M/5 HCl	23.1±1.25	3	4.1±.54	8
0	M/25 HCl	22.9±1.06	4	4.1±.50	8
2	M/5 NaOH	15.5±1.27	14	1.6±.35	12
1	M/25 NaOH	28.2±1.10	1	6.0±.59	6
3	M/5 $LiNO_3$	16.6±2.13	13	3.7±.51	9
0	M/25 $LiNO_3$	24.8±1.13	2	5.9±.45	7
0	M/5 $Ca(NO_3)_2$	18.7±1.06	10	6.2±.34	5
1	M/25 $Ca(NO_3)_2$	20.3±1.04	9	6.6±.48	3
0	M/1 dextrose	20.3± .95	9	6.7±.44	2
0	M/5 dextrose	21.0± .92	7	6.7±.70	2
0	M/5 $NaNO_3$	21.4±1.24	6	8.5±.79	1
0	M/25 $NaNO_3$	20.7± .74	8	6.4±.50	4
0	Distilled H_2O	22.6± .88	5	8.5±.69	1
0	No treatment	18.2±1.36	11	6.7±.45	2
5	Hoagland solution, no Fe	6.1± .95	20	0.9±.26	15
7	Hoagland solution+Fe	6.4± .59	19	0.5±.17	17
4	Fe tartrate	7.9±1.16	17	1.9±.46	11
9	M/25 $KMnO_4$	5.3± .72	22	0.6±.24	16
4	M/5 K_2HPO_4	3.1± .49	23	0.0	18
4	M/25 K_2HPO_4	6.8± .75	18	1.3±.33	14
3	M/5 citric acid	5.7± .52	21	.5±.18	17
3	M/25 citric acid	7.9± .76	17	1.4±.24	13
3	M/5 $(NH_4)_2SO_4$	8.5± .85	16	1.3±.27	14
4	M/25 glycocoll	11.5±1.36	15	2.2±.35	10

It appears from the data given in table 9 that none of the solutions used caused significantly greater total growth than distilled water. $M/5 LiNO_3$ was the only solution which caused visible injury to the cuttings. In this case one or two centimeters of the apex turned brown within a few days. The injury was probably caused by a high concentration of the salt as a result of the evaporation of water from the cut surface.

The treatment with HCl did not affect top growth and only slightly reduced root growth. The sprout growth on cuttings treated with M/5 NaOH was poor, compared with that on cuttings treated with M/25 NaOH. Cross sections made from cuttings treated with the stronger solution of NaOH showed reddish discolorations in the woody part of the cutting through which the solution passed. Within these areas starch was present while none was found in the unaffected regions.

All of the organic compounds used, with the exception of dextrose, retarded growth. There is no indication that dextrose increased either top or root growth. It is of interest to note that Hoagland solution retarded growth although one of its constituents is $Ca(NO_3)_2$ which gave fair results. It is possible that the presence of K_2HPO_4 in Hoagland solution may be the retarding factor since when it was used alone very little growth was produced.

The fact that with some of the compounds the growth produced was poor may be due to interference with the movement of growth-promoting substances. There was some indication that certain compounds hastened twig growth, but the total amount of growth was not increased. None of the substances used had any effect upon the order of bud development.

(*c*) Immature Chinese Lemon Cuttings Treated with Sugar Solutions

Cuttings from which all leaves were removed were taken from the apical part of immature shoots on May 23. Each cutting possessed seven nodes. They were divided into six lots of 18 cuttings each. The solutions used were M/5 and M/10 dextrose, M/5 and M/10 cane sugar and distilled water. One lot served as a control. The cuttings of each lot stood in the solution to a depth of one centimeter for 24 hours without the application of suction. They were then rinsed with distilled water and planted in flats containing river sand. On July 29 the cuttings were removed and the roots and sprouts weighed. The results are summarized in table 10.

The amount of sprouts produced by cuttings treated with distilled water and by the untreated ones was far in excess of that produced by cuttings treated with the various sugar solutions. The amount of roots produced by the cuttings treated with M/10 cane sugar solution compares favorably with that produced by the control cuttings. It will be seen that with all sugar solutions the percentage of the cuttings which died is considerable while none of those treated with distilled water or of the controls died. It is therefore probable that leaving such cuttings in the sugar solutions longer than 24 hours would result in a still greater loss.

TABLE 10

IMMATURE CHINESE LEMON CUTTINGS, MAY, 1924; 18 CUTTINGS IN EACH LOT

Treatment	Average total length of sprouts per cutting, cm.	Average green weight (grams)		Number of cuttings dead
		Sprouts	Roots	
M/5 dextrose	2.3	.28	.12	6
M/10 dextrose	2.1	.22	.10	7
M/5 cane sugar	1.5	.21	.08	7
M/10 cane sugar	2.6	.44	.19	7
Distilled H_2O	3.4	.66	.15	0
Control	3.8	.74	.21	0

Several cuttings similar to those which were treated were examined for starch. In the apical and middle regions, traces of starch were present in the cambium; in the basal part of the cutting starch was also found in the medullary rays.

None of the treatments had the slightest effect upon the order of sprout development. The sprouts from the first and second bud were always the longest and when the third or fourth bud grew out the resulting sprout was very short. There was practically no difference in the number of sprouts produced.

(*d*) DISCUSSION

The results of these experiments show no effect on the order of sprout development or growth on either mature or immature cuttings with any of the substances injected. In the case of M/5 NaOH reduced growth was probably due to the locking up of a part of the food reserve supply. However the injected cuttings started to grow just as quickly as others which produced a greater amount of growth.

The detrimental effect of some of the compounds upon mature cuttings may be explained on the assumption that these compounds interfered with the transformation of food reserves into growth-promoting substances. This view is strengthened by the fact that the release from dormancy of the buds was delayed or entirely prevented. The fact that many of them remained alive for many weeks shows that these compounds as such were not otherwise injurious.

The retarding effect of Hoagland solution is of interest. It contains both $Ca(NO_3)_2$, which is not detrimental, and K_2HPO_4, which is detrimental to the production of sprouts.

V. BEHAVIOR OF HORIZONTAL LEMON SHOOTS

This part of the investigation is an attempt to study the factors involved in the distribution of sprouts on horizontal shoots and cuttings.

All material was obtained from trees growing on the grounds of the Citrus Experiment Station, Riverside, California. The experiments in the orchard were carried out with shoots of the Eureka lemon and those in the laboratory with Chinese lemon cuttings.

With upright lemon shoots are cut back to the mature wood and brought into a horizontal position, sprout development will be confined to the dorsal side with the exception of one or two sprouts which will appear on the ventral side close to the cut. Cuttings of the Chinese lemon grown in a horizontal position behave similarly.

It is often claimed that by bending a shoot into a horizontal position the tissues at the bend are compressed and thus impede the flow of sap to the buds on the ventral side. This view is not supported by the facts. In the first place no tissues are compressed when cuttings are grown horizontally and yet sprouts have not been seen to occur on the ventral side. Secondly, lemon shoots always exhibit this behavior whether the tissues are compressed or stretched. A long shoot bent into a U-shape and tied so that both arms lie in the same plane develops sprouts only on the dorsal side of each arm. Figure 8 shows two Chinese lemon plants both of which were bent in the same way. One plant was grown upside down so that compressed tissues were on the dorsal side and the other plant remained in the normal position. It will be seen that in both cases growth was distributed along the dorsal side.

Light is apparently not a factor of primary importance in initiating sprout growth of lemon shoots or cuttings. Chinese lemon cuttings suspended horizontally in saturated air produced sprouts only on the dorsal side in light as well as in darkness. This is also true when the dorsal side is darkened and the ventral side illuminated.

Loeb[15] suggested that the sprouts on the dorsal side of a horizontal shoot may inhibit growth on the ventral side. He was successful in inducing outgrowth on the ventral side of a horizontally suspended stem of *Bryophyllum calycinum* when he removed the buds on the dorsal side. No evidence of this inhibiting action could be obtained with lemon shoots. Cuttings were split longitudinally and either suspended in saturated air or placed in moss or sand. Whenever the halves of the cuttings were placed so that the buds were on the ventral side, no growth occurred, but when they were placed in the reverse position sprouts developed promptly. It was observed that the growth from a cutting divided in this manner was weaker than that made by whole stems.

Destroying the buds on the dorsal side of either cuttings or horizontal shoots had no effect on the buds of the ventral side but as a result of this operation many cuttings and shoots produced sprouts from adventitious buds on the dorsal side.

On several horizontal shoots no dorsal sprouts were allowed to grow for three years, yet none of the buds on the ventral side, with the exception of one or two close to the apex, showed any signs of growth.

The influence of position upon the development of sprouts can be shown in other ways. When a horizontal cutting is allowed to develop sprouts from the dorsal side and is then revolved through an arc of 180 degrees, the buds from the previously ventral side then produce sprouts while the original sprouts gradually cease growing and in some cases die.

In order to study this behavior quantitatively the following experiment was made. Forty one-year-old vertical lemon shoots were cut back to the mature wood and bent into a horizontal position before growth started in the spring. Twenty of these shoots remained in this position during the entire season, while the twenty others were bent in the opposite direction four weeks after the appearance of sprouts when the average length of the sprouts was 5.4 cm.

In less than two weeks the buds which were previously on the ventral side became active and by the end of the growing season the average length of sprouts per mother shoot was 15.1 cm., as compared

with 12.6 cm. for the sprouts which were originally on the dorsal side of the reflexed shoots. This shows that though the original sprouts were not suppressed by the new set the latter exceeded the former in spite of a later start.

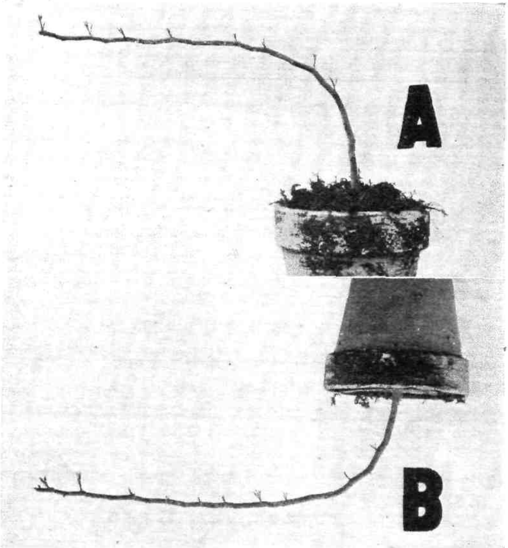

Fig. 8. *Chinese lemon shoots bent into a horizontal position.* (*a*) Right side up; (*b*) upside down.

The average length of sprouts produced by the reflexed shoots, 27.7 cm., compares favorably with that produced by horizontal control shoots, which was 24.6 cm. In total growth there is practically no difference between the two sets of shoots. In centimeters of length of sprouts per 100 sq. cm. of shoot area, the reflexed shoots produced 66 cm. and the control 76 cm. The average number of sprouts on the former shoots was twelve and on the latter seven. In other words, the total amount of growth is not much influenced by the number

of sprouts produced. The only obvious difference was that where fewer sprouts were produced their length was greater.

It has already been said that the seasonal growth of lemon sprouts is made in three cycles. The growth on 225 horizontal mother shoots as determined by measuring the total increments of all sprouts was as follows: 1st cycle, 181 cm., 2nd cycle, 77 cm., and 3rd cycle, 71 cm.

It will be remembered that the original set of sprouts averaged 5.4 cm. when the position of the shoot was changed, and that they reached an average length of 12.6 cm. This corresponds to the average length of 11.5 cm. of sprouts produced by the control shoots during the 1st cycle. It is evident that the change in position did not influence the growth of the down-pointing sprouts until the completion of the 1st cycle, while the sprouts on the dorsal side continued their growth to the end of the season.

These results suggest that gravity may be one of the factors responsible for the distribution of growth on horizontal shoots. For the purpose of studying the effect of gravity the following experiments were made.

Several dormant Chinese lemon cuttings were suspended horizontally in saturated air and revolved through an arc of 180 degrees once every week. Sprouts appeared on both sides within ten days and the rate of growth was approximately the same.

Another set of dormant Chinese lemon cuttings were revolved continuously in a moist atmosphere. This resulted in growth from all sides, and the sprouts were confined to the apical portion of the cutting. When the motor was stopped for about two weeks the sprouts which happened to be on the dorsal side continued to elongate; if kept in this position long enough some of the sprouts pointing downward deteriorated.

From these observations it seems that gravity is a controlling factor in the distribution of sprout growth. The cuttings revolved through an arc of 180 degrees once every week produced sprouts which were in the same plane. By revolving the cuttings continuously the force of gravity was equalized and consequently growth occurred on all sides.

(*a*) Effect of Notching on Outgrowth of Buds

Unbranched dormant lemon shoots were cut back to the mature wood and tied in a horizontal position. From two to four buds on either the dorsal or the ventral side of each shoot were treated in

the manner described below. For each treatment twenty-five shoots were used.

1. A slanting cut was made, beginning about 1 cm. above the bud and extending at an angle of about 20 degrees into the wood to a point 1 cm. below the bud, thus severing the bud from the mother shoot, except on the lower side. A piece of mica was inserted to keep the cut from healing over.
2. A cut was made as above but beginning below the bud.
3. A notch was made above the bud, removing a small crescent-shaped piece of bark.
4. A notch was made as in the last case but below the bud.

The results are given in table 11. It is evident that buds grow if a notch or cut is made above the bud but that sprout growth is inhibited when a notch or cut is made below.

TABLE 11

HORIZONTAL LEMON SHOOTS; SHOWING THE EFFECT OF NOTCHING UPON SPROUT GROWTH

Treatment	Number of buds treated	Per cent of buds which grew	Average total length of sprouts (cm.) per 100 sq. cm. of shoot area
Dorsal buds:			
1	70	98.6	103
2	68	1.5	96
3	71	91.6	111
4	61	9.8	116
Ventral buds:			
1	80	73.3	104
2	71	0.0	109
3	70	51.4	112
4	77	1.3	101
Controls	....	....	106

In order to determine whether the age of the bud on the mother shoot is a factor, buds of the same age were inserted on both dorsal and ventral sides of shoots. These inserted buds were notched in the manner previously described and the results were identical. An attempt was also made to force out buds on the ventral side of the shoot by one longitudinal notch made on each side of the bud; but the results were negative.

It was observed that the twigs produced on the ventral side as a result of notching made the first cycle of growth only. Furthermore several attempts to induce twig growth on the ventral side by notching or cutting later in the season failed.

In the discussion of vertical shoots data were presented to show that the amount of growth produced depends upon the size of the mother shoot. The last column in table 11 shows that this also holds for horizontal shoots.

Although these values agree closely among themselves yet they are only about one-half as large as those obtained with vertical shoots (table 6). But the two cases are entirely different. For the vertical shoots the values given represent the entire shoot whereas in the horizontal shoots only a portion of the entire shoot is represented. In bending a lemon shoot, only about one-half of it can be brought into a horizontal position while the other part remains more or less in a vertical position. This latter portion of the shoot produces the most vigorous growth especially on the convex side of the bend. The growth of these sprouts if left undisturbed retards the development of the sprouts on the horizontal part of the shoot.

Dormant Chinese lemon cuttings contain an abundance of starch but the few sprouts produced on the dorsal side, when grown in a horizontal position, apparently are able to utilize it since the starch has disappeared entirely when the sprouts have reached their maximum length. Sprouts could not be forced out on the ventral side by preventing sprout growth on the dorsal side. If, however, on such shoots a few of the buds on the ventral side were notched above, growth took place from these buds provided the notching was done early in the season.

(*b*) Physical Characteristics of Sap from Dorsal and Ventral Bark

Before the beginning of the growing season succulent, one-year-old lemon shoots were cut back to the mature wood and bent into a horizontal position. The horizontal portion of these shoots varied from 50 to 100 cm. in length. In May, when the sprouts on the dorsal side averaged a few centimeters in length, two sets of samples of 7 to 10 shoots were taken. This was repeated once every month throughout the growing season. One set was taken about 2 P.M. and the other before sunrise. The bark of the dorsal and of the ventral sides

were separated and thoroughly frozen. The material was then ground up in a meat chopper and the sap expressed with a hand press. The *freezing point, the hydrogen-ion concentration* and the *viscosity* of the sap were determined.

For the freezing-point determination the ordinary Beckmann apparatus was used. The depression of the freezing point as read from the thermometer was corrected for under-cooling by the formula given by Harris and Gortner.[9]

The per cent of total solids was determined by means of a refractometer according to the method employed by Gortner and Hoffman.[8] The viscosity was measured with an Ostwald viscosimeter at a temperature of 25° C. The P_H value was determined by comparing samples of sap with standard buffer solutions. The results are summarized in table 12 and presented graphically in figures 9, 10 and 11.

TABLE 12

Characteristics of Sap from Horizontal Lemon Shoots. Upper Figures for Each Date Pertain to Dorsal Side of Shoot and Lower Figures to Ventral Side

Date	Samples taken in afternoon				Samples taken in morning			
	Δ	Total solids (%)	Viscosity (H_2O = 100) (seconds)	P_H	Δ	Total solids (%)	Viscosity (H_2O = 100) (seconds)	P_H
May 1	1.224			5.9	1.117			5.8
	1.204			5.8	1.153			5.9
June 6	.982	14.60		5.6	.898	12.50		5.7
	1.005	14.00		5.7	.938	13.10		5.6
July 9	1.067	16.80	190	5.5	.927	15.30	165	5.6
	1.082	16.80	180	5.5	.925	15.30	160	5.7
Aug. 6	1.066	18.40	200	5.5	.918	15.45	170	5.6
	1.056	17.75	195	5.5	.889	15.25	170	5.6
Sept. 25	1.208	21.00	230	6.0	1.043	17.65	195	6.1
	1.217	20.40	220	5.9	.999	16.40	175	6.0
Oct. 25	1.252	21.40	310	5.8	1.140	19.60	230	5.8
	1.221	20.80	215	5.8	1.089	18.05	200	5.8

Refractometer and Viscosimeter were not available during the early part of the investigation.

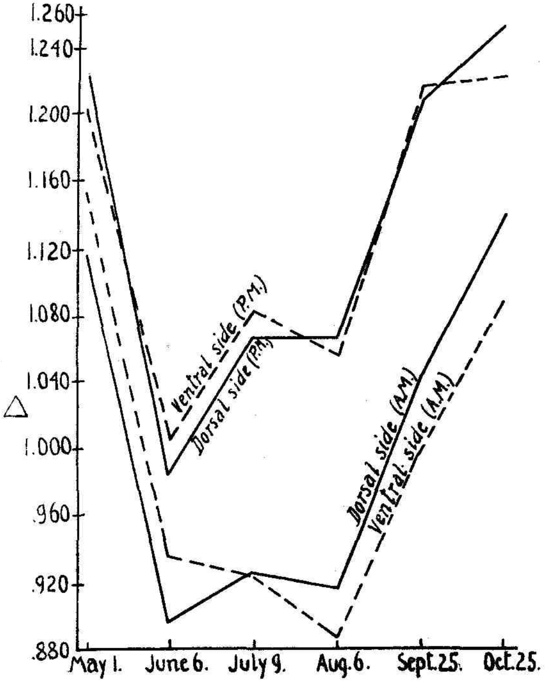

Fig. 9. Horizontal Eureka lemon shoots. Freezing-point depression of sap of dorsal and ventral side.

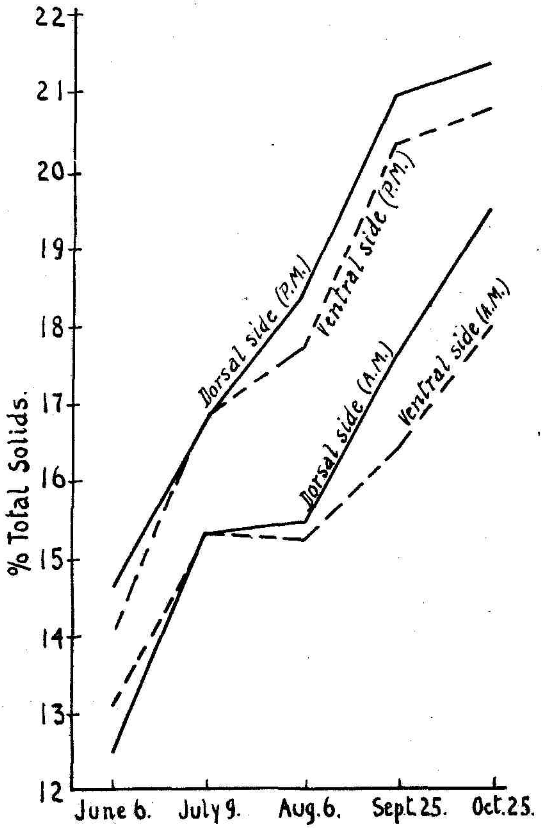

Fig. 10. Horizontal Eureka lemon shoots. Per cent of total solids in sap of dorsal and ventral side.

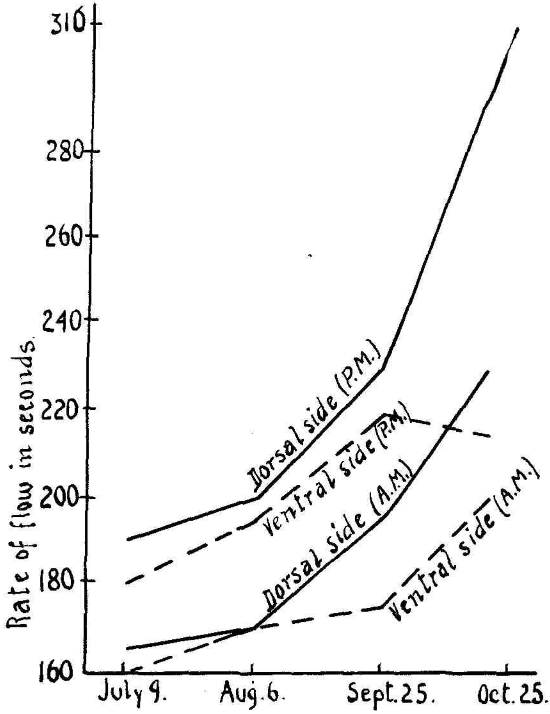

Fig. 11. Horizontal Eureka lemon shoots. Viscosity of sap of dorsal and ventral side compared with rate of flow of water taken as 100.

It is apparent that the freezing-point depression of the sap of the afternoon samples showed practically no difference except in October, when the sap of the ventral side was less concentrated. In the morning samples the ventral side was more concentrated until July, while during the rest of the growing season the reverse is the case.

The curve of total solids follows in a general way that of the freezing-point depression. However, in the case of the afternoon samples, the per cent of total solids is consistently greater on the dorsal side except in July, when the dorsal and ventral sides were alike. The morning samples gave similar results except that the order was reversed during June.

The difference in viscosity between dorsal and ventral sides was small during the early part of the season but it was very marked during October.

The P_H values fluctuated so little that graphs are unnecessary.

The most important point to be considered in the interpretation of these data is, whether any of these methods furnish sufficient evidence that the difference in behavior between the dorsal and the ventral side of horizontal lemon shoots can be related to differences in the sap during the initial period of growth. The determined differences in freezing-point depression and total solids, early in the growing season, were small and inconsistent; we cannot conclude from these data, therefore, that an actual general difference between dorsal and ventral sides existed.

It is interesting to note the great differences in sap concentration between afternoon and morning samples. Sap from leaves and from the bark of the trunk also show this constant difference.

Since none of the data obtained by the various methods mentioned indicate a difference in the sap of dorsal and ventral side early in the season, it was thought that there may be a difference in enzymatic activity. The studies of Heinicke[11] suggest that the catalase activity of apple leaves and bark may be taken as one of the indicators of the nutritive or physiological condition of those tissues. A series of determinations were made in the manner described by Heinicke,[11] with the bark of dorsal and ventral sides of shoots similar to those used for sap studies.

The results are summarized in tables 13 and 14. It will be seen (table 13) that, although the differences are not great, yet they are constant. *Until about August 20 the catalase activity was greater* in the dorsal than in the ventral side of these shoots. It will be noted *that this was true whether the shoots were in full sunlight or in the* shade. The samples taken August 25 and September 15 showed a greater catalase activity on the ventral side. This difference in favor of the ventral side becomes very large as the shoots become older (table 14).

TABLE 13

RELATIVE CATALASE ACTIVITY OF DORSAL AND VENTRAL BARK OF LEMON SHOOTS WHICH WERE BENT INTO A HORIZONTAL POSITION IN THE SPRING OF 1924. SAMPLES TAKEN BETWEEN 7:30 AND 10 A.M.

Date (1924)	Position of shoot	Tissue	Seconds required to liberate 1–5 cc. of oxygen				
			1	2	3	4	5
June 25	Full sunlight	Dorsal	10	20	30	60	95
		Ventral	10	20	40	75	125
July 14	Partly shaded	Dorsal	7	15	30	60	95
		Ventral	7	15	35	65	105
July 14	Shaded	Dorsal	7	15	35	60	95
		Ventral	7	20	45	80	120
July 14	Partly shaded	Dorsal	5	12	20	40	65
		Ventral	7	15	30	55	85
July 23	Full sunlight	Dorsal	7	15	30	60	95
		Ventral	7	20	40	80	125
July 23	Partly shaded	Dorsal	10	25	50	90	120
		Ventral	10	25	55	95	135
Aug. 11	Shaded	Dorsal	7	15	30	55	95
		Ventral	7	15	35	65	115
Aug. 11	Shaded	Dorsal	10	20	35	60	105
		Ventral	10	25	45	70	125
Aug. 18	Full sunlight	Dorsal	7	15	35	60	95
		Ventral	7	15	40	75	110
Aug. 25	Shaded	Dorsal	7	15	35	60	95
		Ventral	7	15	30	55	85
Sept. 15	Full sunlight	Dorsal	7	15	40	75	105
		Ventral	7	15	35	60	90

TABLE 14

RELATIVE CATALASE ACTIVITY OF DORSAL AND VENTRAL BARK OF HORIZONTAL LEMON SHOOTS. SAMPLES TAKEN BETWEEN 7:30 AND 10 A.M.

Date (1924)	Tissue	Seconds required to liberate 1–5 cc. of oxygen				
		1	2	3	4	5
Shoots bent in 1923:						
July 25	Dorsal	7	15	25	45	70
	Ventral	5	10	20	35	55
Aug. 20	Dorsal	7	15	35	65	100
	Ventral	5	10	15	25	45
Shoots bent in 1922:						
Aug. 11	Dorsal	15	45	100	175	255
	Ventral	5	7	15	20	30
Aug. 15	Dorsal	10	30	65	115	170
	Ventral	5	7	10	15	20
Aug. 18	Dorsal	10	25	60	105	160
	Ventral	5	10	17	25	40
Shoots bent in 1920:						
June 23	Dorsal	7	15	30	60	105
	Ventral	5	10	15	20	30
June 25	Dorsal	12	35	85	150	240
	Ventral	5	10	15	20	30
June 26	Dorsal	10	35	70	120	160
	Ventral	5	10	15	30	45
June 28	Dorsal	15	45	105	170	250
	Ventral	5	10	15	20	30

The dorsal bark of these older shoots is much thinner, peels off less readily and has a darker color than the ventral bark.

(c) DISCUSSION

The results obtained with horizontal shoots justify the assumption that the initial period of sprout growth is governed by growth-promoting substances. The growing sprouts on the dorsal side seem to be able to draw on the supply of growth-promoting substances which are contained in the dorsal as well as in the ventral side of the shoot. On this basis we can account for the fact that the reflexed shoots which produced two sets of laterals, produced approximately the same amount of sprout growth as shoots which remained in the original horizontal position.

Notching above a bud on the ventral side affects only that bud, which indicates that if there is a longitudinal movement of growth-promoting substances it is slight. A notch below the bud prevents the growth-promoting substances from reaching that bud. On the dorsal side of the shoot, it is impossible to say whether or not a notch above a bud is responsible for the outgrowth of that bud, because the chances are even that it would have grown out without notching. But a notch below a bud on the dorsal side prevents growth, hence there must also be a slight longitudinal movement along the dorsal side.

The initial period of sprout growth on horizontal shoots is evidently dependent on the supply of stored food reserves. This view is strengthened by the fact that notching on the ventral side, and to some extent on the dorsal side as well, is effective only until the stored food reserves are exhausted.

With the methods employed the plant juices obtained from the dorsal and ventral sides do not show a difference in their physical properties. The data on catalase activity do not indicate that the enzyme is a factor in the initiation of sprout growth. If the small but constant differences in favor of the dorsal side of younger shoots (table 13) were significant, then we would expect sprout growth on the ventral side in older shoots because in this case the order is reversed (table 14). The reason for the great difference in catalase activity in older shoots is due to the fact that cambial activity is practically confined to the ventral side. A cross section shows this eccentric growth very clearly.

It is not clear how the initiation of sprout growth can be explained on the basis of Child's theory. We can say that by bending a shoot into a horizontal position a new metabolic gradient was set up, otherwise it would behave like a vertical shoot. But this does not furnish a basis for an explanation. Jones[12] however, obtained results with root cuttings of seakale which are in general accord with Child's conception of "metabolic gradients."

We may now consider the possibility of an inhibitory substance being responsible for the initiation of sprout growth on the dorsal side. According to this concept the inhibitory substance is produced by the growing sprouts. As in the case of vertical dormant shoots, the chief objection is that during the initial period, the sprouts are either absent or, if present, they are too small to produce enough of this substance to inhibit growth all along the ventral side. It will be recalled that on some shoots the dorsal side was kept free from

sprouts for three years, yet no growth resulted on the ventral side. If we assume that there is a supply of the inhibitor in the dormant shoot which, as the shoot is bent, settles to the ventral side, it is difficult to see how this limited supply can keep the ventral side dormant for such a long time.

Furthermore, when a horizontal shoot, bearing sprouts on the dorsal side, was bent in the opposite direction, a new set of sprouts developed from the dormant buds of the previously ventral side. This new set of sprouts is unable to retard the growth of the original set throughout the initial period.

If we consider the second period of growth in contrast to the initial one, however, we find that the sprouts at the apex continue to elongate somewhat while those further back generally fail to produce the second growth cycle. We may assume that the apical sprouts produce an inhibitory substance which is responsible for this condition. But this influence is not so pronounced as in vertical shoots. When a shoot is bent, part of it remains more or less vertical. The bend does not affect the growth during the initial period, yet during the later period the rate of sprout growth at or immediately below the bend is far greater than that on the horizontal portion. As the shoot becomes older the horizontal part increases in diameter at a slower rate than the vertical part and finally ceases its growth.

VI. SUMMARY

1. The investigations here reported deal with the factors which govern the initiation of sprout growth on vertical and horizontal Eureka lemon shoots and Chinese lemon cuttings.

2. When a vertical lemon shoot is cut back to the mature wood, sprouts are produced only from the uppermost buds. The length of the sprouts decreases from the apex downward.

3. On vertical shoots, buds normally dormant can be released from dormancy by various mechanical means such as notching or girdling above a bud or wrapping tape around the upper portion of the shoot.

4. The amount of sprout growth produced is in proportion to the size of the shoot or cutting.

5. The temporary taping of the upper portion of a cutting divides it into two physiological units, each of which produces sprout growth in proportion to the size of the piece. There is no indication that the

sprouts on one portion inhibit the growth of sprouts on the other. This is also true of attached lemon shoots during the initial period of growth, but in the later growth the apical sprouts may slow up the rate of growth of subapical sprouts.

6. A dormant mature shoot or cutting contains stored food reserves in proportion to the size of the piece. With proper temperature and moisture conditions the food reserves are transformed into growth-promoting substances. This transformation is probably a gradual one which begins at the apex. When the uppermost buds are taped the supply of growth-promoting substances in that region does not become available to the growing subapical sprouts. But these sprouts are able to draw on the supply of growth-promoting substances from below.

7. This transformation of food reserves into growth-promoting substances would account for the initial period of sprout growth. The apical dominance exhibited during the later growth period may be explained on the assumption that an inhibitory substance is produced by the growing shoots which inhibits further elongation of subapical sprouts.

8. Injecting various chemical compounds into dormant Chinese lemon and Ligustrum cuttings did not affect apical dominance. Furthermore none of the substances increased the normal amount of sprout growth. Introducing cane sugar and dextrose solutions into immature Chinese lemon cuttings also gave negative results.

9. Horizontal lemon shoots or Chinese lemon cuttings produce sprouts on the dorsal side only. By bending a horizontal shoot in the opposite direction a new set of sprouts is produced from buds which would otherwise have remained dormant. By this method the total number of sprouts is practically doubled, but the total amount of growth produced is approximately the same as that produced by shoots which remained in the original horizontal position.

10. Notching above a bud on the ventral side results in sprout growth from that bud. This operation is effective only during the early part of the growing season. A notch below a bud either on the dorsal or ventral side is not effective.

11. No growth takes place on the ventral side when the buds on the dorsal side are burned out, but sprouts will appear from adventitious buds on the dorsal side. Preventing sprout growth on the dorsal side does not cause growth on the ventral side.

12. Compression or tension caused by bending a shoot has no effect upon the distribution of sprouts during the initial period.

13. No differences in the physical properties of the sap of the dorsal and ventral side were found at the beginning of the growing season. The determinations included freezing point, per cent of total solids, viscosity and hydrogen-ion concentration.

14. The relative catalase activity was slightly greater on the dorsal side of younger shoots. As the shoots become older a greater difference exists but the order is reversed.

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